pAcVE.03 Baculovirus Transfer Vector

Cat. No.: 20030 Quantity: 10µg Storage: -20°C

This baculovirus transfer vector integrates the co-expression of a vankyrin gene for increased protein production, a honey bee melittin signal sequence for direct secretion and an optional C-terminal 6xHis-tag for ease of purification.

Description

pAcVE.03 is a baculovirus transfer vector designed for high levels of recombinant gene expression under the control of the *Autographa californica* nucleopolyhedro virus (AcMNPV) polyhedrin promoter. pAcVE.03 is derived from pUC57 and contains the pUC origin of replication and the ampicillin resistance gene for propagation and selection in bacteria. pAcVE.03 is designed for the direct secretion of recombinant proteins utilizing the honeybee melittin signal sequence (Tessier *et al.*, 1991). An optional C-terminal 6xHis-tag is provided for ease of purification. Cloning into pAcVE.03 requires the DNA insert to be in frame with the honeybee melittin signal sequence. Foreign genes may be cloned into the multiple cloning site utilizing the following restriction sites: NcoI; SbfI; XhoI; BstZ17I; BglII; SacII and EagI. Increased recombinant protein production over conventional baculovirus transfer vectors is achieved by the co-expression of the gene of interest with a vankyrin expression cassette (Fath-Goodin *et al.*, 2006). pAcVE.03 contains ORF 603 and ORF1629 sequences and allows recombination with the viral DNA for insertion into the polyhedrin locus. The baculovirus transfer vector is compatible with any baculovirus system that utilizes homologous recombination in insect cells, including *flash*BAC (Oxford Expression Technologies) and Bac-N-Blue (Invitrogen).

Contents

The plasmid DNA is dissolved in TE buffer (10mM Tris-HCl, 1 mM EDTA pH7.5). pAcVE.03 baculovirus transfer vector is provided at 10 µg in 50 µl.

Storage

The transfer vector should be placed at -20° C for long- term storage.

Handling

For expression of recombinant protein under the polyhedrin promoter, the gene of interest should be ligated into an appropriate restriction site in the multiple cloning site of the pAcVE.03 vector and should be in frame with the honey bee melittin signal sequence and the C-terminal 6xHis-tag if desired (see diagram and table). In that instance the stop codon needs to be omitted. Transform the recombinant pAcVE.03 transfer vector in *E. coli* cells (DH5α, Top10 or any other suitable strain), propagate the cells under ampicillin selection and purify the recombinant plasmid using standard plasmid purification protocols. For construction of recombinant AcMNPV perform a co-transfection of the purified recombinant pAcVE.03 vector with linearized baculovirus DNA suitable for homologous recombination in insect cells.

Restriction Enzymes Suitable for In-Frame Cloning

pAcVE.03	NcoI	SbfI	XhoI	BstZ17I	Bgl II	SacII	EagI
NcoI	*		*		*	*	*
SbfI			*		*	*	*
XhoI	*	*	*		*	*	*
BstZ17I				*			
Bgl II	*	*	*		*	*	*
SacII	*	*	*		*	*	*
EagI	*	*	*		*	*	*

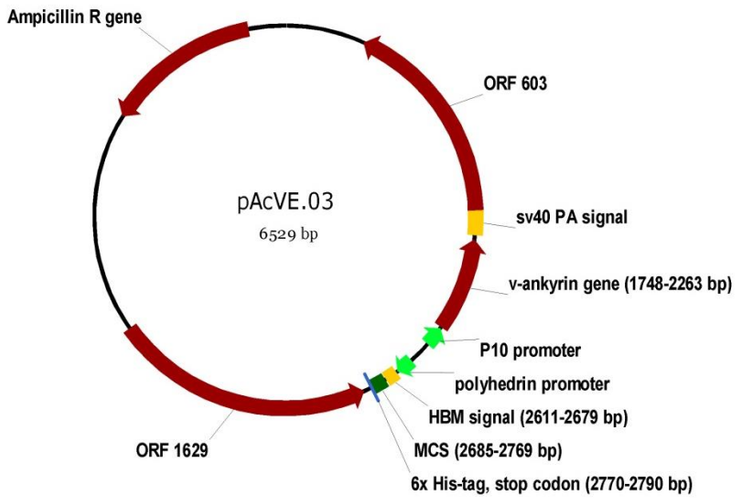
Unique Restriction Sites

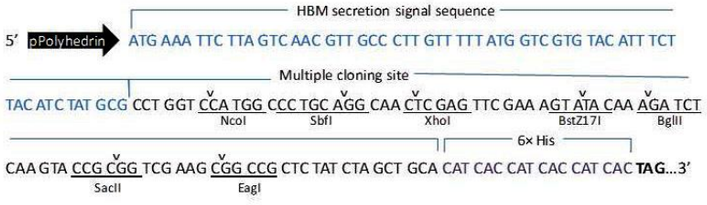
AgeI AleI AlwNI ApaI AvrII BamHI BbvCI BclI BglII BmgBI BplI BpmI Bpu10I BsaAI BsaBI BseRI BseYI BstAPI BstXI BstZ17I EagI Eco53kI EcoNI EcoRI HindIII HpaI KpnI NaeI NcoI NdeI NgoMIV NotI NruI PciI PfoI PpuMI PspOMI SacI SacII SapI SbfI SgrAI SmaI SnaBI SpeI SphI StuI SwaI XbaI XhoI XmaI

Absent Restriction Sites

AscI AsiSI BlpI BmtI BsiWI BspEI BspMI BssHII BstEII Bsu36I DraIII FseI FspAI MscI NheI PflMI PmeI PmlI PshAI RsrII SanDI SexAI SfiI Tth111I XcmI

pAcVE.03 Vector Map and Multiple Cloning Site





ParaTechs' Related Products

Product Name	Catalog No.
pAcVE.01	20010
pAcVE.02	20020
VE Insect Cell Line 01	10010
VE Insect Cell Line 02	10020
VE Insect Cell Line 03	10030

Contract service for recombinant virus construction is available upon request. For further information on other ParaTechs products contact our Technical Services at info@paratechs.com or call (859) 317-9213. See also our web-site at www.paratechs.com.

References

Fath-Goodin, A., Kroemer, J.A., Martin, S.B., Reeves, K., and Webb, B.A. (2006). Polydnavirus genes that enhance the Baculovirus Expression Vector System. Advances in Virus Research, vol. 68, pp. 75-90.

Tessier, D.C., Thomas, D.Y., Khouri, H.E., Laliberté, F., and Vernet, T. (1991). Enhanced secretion from insect cells of a foreign protein fused to the honeybee melittin signal peptide. Gene, 98: 177-183.

This product is intended for research purposes only.

CAUTION: Not intended for human or animal diagnostic or therapeutic uses. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of ParaTechs Corporation is strictly prohibited.

LIMITED WARRANTY

ParaTechs warrants that, at the time of shipment, the Product will conform to the specifications that accompany the Product. This warranty limits ParaTechs liability to replacement of the Product. PARATECHS MAKES NO OTHER WARRANTIES, EXPRESS OR IMPLIED, WITH RESPECT TO THE PRODUCT; INCLUDING ANY WARRANTIES OF MECHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE OR THAT THE PRODUCT DOES NOT INFRINGE ANY PROPRIETARY RIGHTS OF ANY THIRD PARTY.

Product Use Limitation

The pAcVE.03 baculovirus transfer vector ("Product") was developed in collaboration by scientists at ParaTechs and the University of Kentucky Lexington for expression of recombinant proteins. One patent owned by the University of Kentucky Lexington covers components of the Product.

ParaTechs Corporation has an exclusive license to sell the Product to scientists for **academic research or one year commercial evaluation only**, under the terms described below. Use of the Product for any Commercial Purpose (as defined below) other than evaluation requires the user to obtain a commercial license as detailed below. Before using the Product, please read the terms and conditions set forth below. Your use of the Product shall constitute acknowledgement and acceptance of these terms and conditions. If you do not wish to use the Product pursuant to these terms and conditions, please contact ParaTechs Technical Service Department to return the unused and unopened Product for full credit.

ParaTechs grants the purchaser a non-exclusive license to use the enclosed Product for academic research or for commercial evaluation purposes only. The Product is being transferred to you in furtherance of, and reliance on, such license. You may not use the Product, or the materials contained therein, for any Commercial Purpose without a license for such purpose from ParaTechs Corporation.

Commercial Purpose include any use of Product in a Commercial Product, the manufacture of a Commercial Product, any resale of the Product, any use (other than evaluation) of Product to facilitate or advance research or development of a Commercial Product, and any use (other than evaluation) of the Product to facilitate or advance any research or development program the results of which will be applied to the development of Commercial Products.

"Commercial Product" means any product intended for commercial use.

Access to the Product must be limited solely to those officers, employees and students of your entity who need access to perform the aforementioned research or evaluation. Each such officer, employee and student must be informed of these terms and conditions and agree in writing, to be bound by same. You may not distribute the Product to others. You may not transfer modified, altered, or original material from the Product to a third party without written notification to and written approval from ParaTechs.

Inquiries for commercial use should be directed to agoodin@paratechs.com.

Patent information: United States Patent 7,629,160

March 2014